Appl. No: 09/467,076

Amendment dated June 29, 2004

Reply to Office Action of April 21, 2004

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:** 

1. (Currently Amended) A method of producing ungulate embryonic stem-like

cells, wherein said cells comprise a nucleus derived from an adult differentiated cell of a first

ungulate species and mitochondria from an oocyte of a second ungulate species other than the

species of said adult differentiated cell, comprising the following steps:

) inserting a donor differentiated cell or cell nucleus of said first ungulate species

into a recipient animal oocyte of said second ungulate species under conditions suitable for the

formation of a nuclear transfer (NT) unit, wherein the endogenous oocyte nucleus is removed or

inactivated before, concurrent, or after introduction of donor cell or nucleus;

(ii) activating the resultant nuclear transfer unit;

(iii) additionally inserting into said oocyte cytoplasm derived from a second oocyte or

a blastomere of the same species as the donor cell or nucleus;

(iv) culturing said activated nuclear transfer unit until greater than the 2-cell

developmental stage;

(v) dissociating said activated nuclear transfer unit; and

(vi) isolating cells having the nuclear material of said first ungulate species and the

mitochondria of said second different ungulate species from said disassociated nuclear transfer

unit to obtain embryonic stem-like cells.

2. (Previously amended) The method of claim 1, which further includes introducing

the mitochondrial DNA of the same species as the donor cell or nucleus into the recipient oocyte.

3. (Original) The method of Claim 1, wherein said cytoplasm is introduced before,

concurrent, or after introduction of donor cell or nucleus.

4. (Currently amended) The method of claim 4 3, wherein said introduction occurs

within about six hours of introduction of the donor cell or nucleus.

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- 5. (Previously amended) The method of Claim 4, wherein said second oocyte is an immature oocyte.
- 6. (Previously amended) The method of Claim 5, wherein said second oocyte is an immature bovine oocyte.
- 7. (Previously amended) The method of Claim 5, wherein said immature oocyte is matured *in vitro* prior to isolation of cytoplasm thereform.
- 8. (Previously amended) The method of Claim 5, wherein said immature oocyte is activated *in vitro* prior to isolation of cytoplasm therefrom.
- 9. (Original) The method of Claim 8, wherein said *in vitro* activation comprises contacting said oocyte with a compound that increases calcium levels.
- 10. (Previously amended) The method of Claim 2, wherein all or part of the cytoplasm of the recipient oocyte is removed prior to introduction of cytoplasm from said at least one second oocyte or blastomere of the same species as the donor cell or nucleus.
- 11. (Previously amended) The method of Claim 1, wherein the cell or cell nucleus inserted into the enucleated oocyte is a bovine cell.
- 12. (Previously amended) The method of Claim 11, wherein said bovine cell is an adult cell.
- 13. (Previously amended) The method of Claim 11, wherein said bovine cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

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- 14. (Previously amended) The method of Claim 11, wherein the recipient oocyte is obtained from a bovine mammal.
- 15. (Previously amended) The method of Claim 14, wherein the animal oocyte is obtained from Bos taurus.
- 16. (Previously amended) The method of Claim 1, wherein said first and second ungulate species are both of an ungulate that is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.
- 17. (Original) The method of Claim 1, wherein the enucleated oocyte is matured prior to enucleation.
- 18. (Previously amended) The method of Claim 1, wherein the fused nuclear transfer unit is activated *in vitro*.
- 19. (Previously amended) The method of Claim 1, wherein the activated nuclear transfer unit is cultured on a feeder layer culture.
- 20. (Original) The method of Claim 19, wherein the feeder layer comprises fibroblasts.
- 21. (Original) The method of claim 1, wherein in step (v) cells from a NT unit having 16 cells or more are cultured on a feeder cell layer.
- 22. (Original) The method of Claim 21, wherein said feeder cell layer comprises fibroblasts.

- 23. (Original) The method of claim 22, wherein said fibroblasts comprise mouse embryonic fibroblasts.
- 24. (Previously amended) The Method of Claim 1, wherein the resultant embryonic stem-like cells are induced to differentiate.
- 25. (Previously amended) The method of Claim 11, wherein the resultant bovine embryonic stem-like cells are induced to differentiate.
  - 26. (Original) The method of Claim 1, wherein fusion is effected by electrofusion.
- 27. (Currently amended) Ungulate embryonic stem-like cells obtained according to the method of Claim 1, which cells have mitochondria of said second ungulate species.
- 28. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 11, which cells have mitochondria of said second ungulate species.
- 29. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 12, which cells have mitochondria of said second ungulate species.
- 30. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 13, which cells have mitochondria of said second ungulate species.
- 31. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 14, which cells have mitochondria of said second ungulate species.
- 32. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 15, which cells have mitochondria of said second ungulate species.

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- 33. (Previously amended) Differentiated bovine cells obtained by the method of Claim 25, which cells have mitochondria of said second ungulate species.
- 34. (Previously amended) The differentiated bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells, which cells have mitochondria of said second ungulate species.

## 35. (Canceled)

- 36. (Previously amended) The method of Claim 1, further comprising a step whereby a desired gene is inserted, removed or modified in said embryonic stem-like cells.
- 37. (Original) The method of Claim 36, wherein the desired gene encodes a therapeutic enzyme, a growth factor or a cytokine.
- 38. (Previously amended) The method of Claim 37, wherein said embryonic stem-like cells are bovine embryonic stem-like cells.
- 39. (Original) The method of Claim 36, wherein the desired gene is removed, modified or deleted by homologous recombination.
- 40. (Original) The method of Claim 1, wherein the donor cell is genetically modified to impair the development of at least one of endoderm, ectoderm and mesoderm.
- 41. (Original) The method of Claim 1, wherein the donor cell is genetically modified to increase differentiation efficiency.
- 42. (Original) The method of Claim 40, wherein wherein the cultured nuclear transfer unit is cultured in a media containing at least one caspase inhibitor.

- 43. (Original) The method of Claim 1, wherein the donor cell expresses a detectable label that is indicative of the expression of a particular cyclin.
- 44. (Original) The method of Claim 40, wherein the donor cell has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.
- 45. (Original) The method of Claim 41, wherein said donor cell has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.
- 46. (Original) The method of Claim 45, wherein said gene or genes are operably linked to a regulatable promoter.
- 47. (Original) The method of Claim 1, wherein the donor cell has been genetically modified to inhibit apoptosis.
- 48. (Original) The method of Claim 47, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, Gim<sub>L</sub>, Bid, EGL-1, Bcl-CL, Bcl-w, Mcl-1, A1, Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.
- 49. (Original) The method of Claim 48, wherein at least one of said genes is operably linked to an inducible promoter.
- 50. (Previously amended) An ungulate somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is operably linked to a promoter that regulates the expression of a particular cyclin.

- 51. (Original) The cell of Claim 50, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A and H.
- 52. (Original) The cell of Claim 50, wherein the detectable marker is a fluorescent polypeptide.
- 53. (Original) The cell of Claim 52, wherein said mammalian cell is selected from the group consisting of human, primate, rodent, ungulate, canine, and feline cells.
- 54. (Original) The cell of Claim 50, wherein said cell is a human, bovine or primate cell.
- 55. (Previously amended) The method of claim 1, wherein said cells isolated from said disassociated nuclear transfer unit are isolated from cells originating from the inner-most portion of said nuclear transfer unit.
- 56. (Previously amended) An ungulate embryonic stem-like cell isolated from the inner-most portion of a nuclear transfer unit according to the method of claim 55, which cell has mitochondria of said second ungulate species.
- 57. (Previously amended) Differentiated bovine cells obtained by the method of Claim 33, wherein said differentiated cells contain and express and inserted gene.
- 58. (Previously amended) The differentiated bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.